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Recent Advances on the Nutritional Effects Associated with the Use of Garlic as a Supplement

A Historical Perspective on Garlic and Cancer[1]

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ABSTRACT Epidemiological and laboratory studies provide insight into the anticarcinogenic potential of garlic and its constituent compounds. Both water- and lipid-soluble allyl sulfur compounds are effective in blocking a myriad of chemically induced tumors. Part of the protection from these compounds probably relates to a block in nitrosamine formation and metabolism. However, blockage in the initiation and promotion phases of the carcinogenicity of various compounds, including polycyclic hydrocarbons, provide evidence that garlic and its constituents can alter several phase I and II enzymes. Their ability to block experimentally induced tumors in a variety of sites including skin, mammary and colon, suggests a general mechanism of action. Changes in DNA repair and in immunocompetence may also account for some of this protection. Some, but not all, allyl sulfur compounds can also effectively retard tumor proliferation and induce apoptosis. Changes in cellular thiol and phosphorylation stains may account for some of these antitumorigenic properties. The anticarcinogenic potential of garlic can be influenced by several dietary components including specific fatty acids, selenium, and vitamin A. Since garlic and its constituents can suppress carcinogen formation, carcinogen bioactivation, and tumor proliferation it is imperative that biomarkers be established to identify which individuals might benefit most and what intakes can occur with ill consequences.. J. Nutr. 131: 1027S–1031S, 2001.

KEY WORDS: • *garlic* • *allyl sulfur* • *carcinogenesis* • *lipid* • *tumorigenesis*

Garlic has long been revered for its medicinal properties as evidenced by ancient writings from Egypt, Greece, China and India extolling its merits. This reverence has escalated in recent years as a result of the emergence of data indicating that garlic may influence the risk of heart disease and cancer (Fenwick and Hanley 1985, Milner 1996 and 1999, Orekhov and Grunwald 1997, Yoshida et al. 1999). Although significant limitations exist in defining the precise role that garlic has in the cancer process, the likelihood of its significance as a protective agent is supported by both epidemiologic and preclinical studies. Epidemiologic findings about garlic as an anticancer dietary component are presented by Fleischauer and Arab (2001) in this issue. Preclinical studies with cancer models appear to provide some of the most compelling evidence that garlic and related sulfur constituents can suppress cancer risk and alter the biological behavior of tumors.

Experimentally, garlic and its associated sulfur components are reported to suppress tumor incidence in breast, colon, skin, uterine, esophagus and lung cancers (Amagase and Milner 1993, Hussain et al. 1990, Ip et al. 1992, Liu et al. 1992, Shukla et al. 1999, Song and Milner 1999, Sumiyoshi and Wargovich 1990, Wargovich et al. 1988). This protection may arise from several mechanisms including the following: blockage of *N*-nitroso compound (NOC)[2] formation, suppression in the bioactivation of several carcinogens, enhanced DNA repair, reduced cell proliferation and/or induction of apoptosis. It is likely that several of these cellular events are occurring simultaneously and account for the widespread protection that is observed experimentally after garlic supplementation. Nevertheless, it is also apparent that the allyl sulfur compounds in garlic do not function in isolation but are influenced by several components of the diet. Thus, it is not surprising that inconsistencies exist in the literature about the true physiologic importance of garlic as a modifier of the cancer process. This review will focus on evidence that garlic is anticarcinogenic and antitumorigenic and identify some dietary components that should be considered as important variables when assessing the true anticancer potential of garlic.

Nitrosamine formation and bioactivation

Considerable information points to the ability of garlic to suppress the formation of NOC (Atanasova-Goranova et al. 1997, Dion et al. 1997, Kolb et al. 1997, Shenoy and Choughuley 1992). NOC are suspect carcinogens in a variety of

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[2] Abbreviations used: CYP2E1, cytochrome P_{450} 2E1; DADS, diallyl disulfide; DATS, diallyl trisulfide; DMBA, dimethylbenz[*a*]anthracene; GST, glutathione-*S*-transferase; MNU, methylnitrosurea; NOC, *N*-nitroso compounds; SAC, *S*-allyl cysteine.

biological systems and may be a critical environmental factor influencing cancer risk in humans (Brown 1999; Ferguson 1999). Exposure to these potential carcinogens can occur through either ingestion or inhalation of preformed nitrosamines or by the ingestion of their precursors (Lijinsky 1999). A reduction in nitrosamines may occur as a result of the enhanced formation of nitrosothiols after ingestion of garlic or other allium foods. Williams (1983) demonstrated that several sulfur compounds fostered nitrosothiols formation, thereby minimizing the amount of nitrite for NOC synthesis. Studies by Dion et al. (1997) provided evidence that several allium foods contained compounds that were effective in blocking nitrosamine formation. Their studies also documented that not all allyl sulfur compounds were effective in retarding the formation of NOC. *S*-Allyl cysteine (SAC) and its non-allyl analog *S*-propyl cysteine retarded NOC formation, but diallyl disulfide (DADS), dipropyl disulfide and diallyl sulfide were ineffective. These data provide evidence of the critical role that the cysteine residue has in retarding NOC formation (Dion et al. 1997). Because the content of allyl sulfur can vary among preparations, it is likely that not all garlic sources will be equally protective against nitrosamine formation. It should also be pointed out that some of the protection against carcinogenic nitrosamine exposure may occur secondarily to a depression in microbes within the gastrointestinal tract. Mounting evidence indicates that several microorganisms can enhance the synthesis of nitrosoamines. Dion et al. (1997) and many others have provided evidence that several oil-soluble allyl sulfur compounds are effective antimicrobial agents. Thus, the ability of garlic to depress NOC may arise from a number of physiologic events.

Some of the most convincing evidence that garlic is able to reduce nitrosamine formation in humans comes from studies by Mei et al. (1989). They reported that providing 5 g garlic/d completely blocked the enhanced urinary excretion of nitrosoproline arising from the ingestion of supplemental nitrate and proline. The significance of this observation rests on the importance of nitrosoproline excretion as a predictor of the overall capacity for nitrosamine synthesis (Ohshima and Bartsch 1999). Evidence for the importance of this reduction in cancer comes from the ability of garlic to block DNA adducts arising from precursors to a nitrosamine known to induce liver cancer (Lin et al. 1994).

The anticancer benefits attributed to garlic are also consistent with its ability to suppress carcinogen bioactivation. Several publications point to the effectiveness of garlic in blocking DNA aklylation, a primary step in nitrosamine carcinogenesis (Haber-Mignard et al. 1996, Hong et al. 1992, Lin et al. 1994). Consistent with this reduction in bioactivation, Dion et al. (1997) found that both water-soluble SAC and lipid-soluble DADS retarded the mutagenicity of nitrosomorpholine in *Salmonella typhimurium* TA100. Similarly, reduced mutagenicity after aqueous garlic extract exposure has been reported to occur during exposures to ionizing radiation, or treatment with peroxides, adriamycin and *N*-methyl-*N*′-nitro-nitrosoguanidine (Knasmuller et al. 1989).

A block in nitrosamine bioactivation may reflect changes in several enzymes. Cytochrome P_{450} 2E1 (CYP2E1) appears to be one that is particularly vulnerable to the effects of allyl sulfur compounds (Chen et al. 1994, Jeong and Lee 1998, Yang 2001). An autocatalytic destruction of CYP2E1 has been demonstrated and may account for the chemoprotective effects of diallyl sulfide, and possibly other allyl sulfur compounds against some chemical carcinogens (Jin and Baillie 1997). Understanding the variation in the content and overall activity of P_{450} 2E1 would assist in determining who might benefit most from an intervention strategy using garlic or isolated components.

TABLE 1

Cancer causing agents known to be influenced by garlic and/or associated allyl sulfur compounds[1]

Compound	Site	Host
1,2-Dimethylhydrazine	Colon	Rat
3-Methylcholanthrene	Cervix	Mouse
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	Nasal	Rat
7,12 Dimethylbenz[a]anthracene	Mammary	Rat
7,12 Dimethylbenz[a]anthracene	Skin	Mouse
7,12-Dimethylbenz[a]anthracene	Forestomach	Hamster
7,12-Dimethylbenz[a]anthracene	Buccal pouch	Hamster
Aflatoxin B1	Liver	Toad
Aflatoxin B1	Liver	Rat
Azoxymethane	Colon	Rat
Benzo(a)pyrene	Forestomach	Mouse
Benzo(a)pyrene	Lung	Mouse
Benzo(a)pyrene	Skin	Mouse
Benzo(a)pyrene	Bone marrow	Mouse
Methylnitronitrosoguanidine	Gastric	Rat
N-Methyl-*N*-nitrosourea	Mammary	Rat
N-Nitrosodiethylamine	Colon	Rat
N-Nitrosodiethylamine	Nasal	Rat
N-Nitrosodimethylamine	Liver	Rat
N-Nitrosodimethylamine	Nasal	Rat
N-Nitrosodimethylamine	Skin	Mouse
N-Nitrosomethylbenzylamine	Esophagus	Rat
Vinyl carbamate	Skin	Mouse

[1] The magnitude of the response to garlic and/or specific allyl sulfur components depends on the quantity provided, the amount of carcinogen administered and the composition of the diet.

Other carcinogens are also influenced

Allyl sulfur compounds arising from garlic have also been found to effectively block the bioactivation and carcinogenicity of several non-nitrosamines (**Table 1**). The diverse array of compounds and target tissues involved suggests either that garlic or associated constituents have multiple mechanisms of action or, more logically, influence a fundamental step in the overall cancer process. Metabolic activation is a necessary event for many of these carcinogens used in animal studies and possibly for environmental exposures faced by humans. Thus, phase I and II enzymes involved in carcinogen bioactivation and removal may be key in explaining the response to garlic and allyl sulfur compounds. However, few studies have noted significant changes in cytochrome P_{450} 1A1, 1A2, 2B1, or 3A4 activities after supplementation with garlic or related sulfur compounds (Manson et al. 1997, Pan et al. 1993, Wang et al. 1999). Therefore, other enzymes involved in the bioactivation or removal of carcinogenic metabolites may play a role. Singh et al. (1998) provided evidence that the efficacy of various organosulfides to suppress benzo(a)pyrene tumorigenesis correlated with their ability to suppress NAD(P)H:quinone oxidoreductase, an enzyme involved with the removal of quinones associated with this carcinogen. Depressed carcinogen bioactivation because of reduction in cyclooxygenase and lipoxygenase activity may also account for some of the lower incidence of tumors after treatment with some carcinogens (Hughes et al. 1989, Joseph et al. 1994, Liu et al. 1995, McGrath and Milner 1999, Rioux and Castonguay 1998, Roy and Kulkarni 1999). Enhanced glutathione availability and an

elevation in the activity of specific glutathione-*S*-transferase (GST), both factors involved in phase II detoxification, may also be significant in the protection provided by garlic and associated allyl sulfur components. Ingestion of garlic by rats increases the activity of GST in both liver and mammary tissue (Hatono et al. 1996, Manson et al. 1997, Singh and Singh 1997). It should be noted that not all GST isozymes are influenced equally. Hu et al. (1997) provided evidence that the induction of GST pi may be particularly important in the anticarcinogenic properties associated with garlic and allyl sulfur components.

Garlic has also been found to reduce the incidence of tumors resulting from the treatment with methylnitrosurea (MNU), a known direct-acting carcinogen (Lin et al. 1994). Water-soluble SAC (57 μmol/kg diet) and lipid-soluble DADS cause a comparable reduction in MNU-induced O^6-methylguanine adducts bound to mammary cell DNA (Schaffer et al. 1996). Most recently, SAC was not found to provide protection against MNU-induced mammary tumors (Cohen et al. 1999). The reason for this discrepancy is unknown but may relate to the quantity of lipid in the diet or the quantity of carcinogen provided. If there truly are effects of DADS and SAC on MNU carcinogenesis, the mechanism(s) by which it brings about this effect are likely related to changes in DNA repair and/or cell signaling because this is a direct-acting carcinogen. It is possible that garlic could influence mammary gland terminal end bud formation and/or cause a change in rates of DNA repair. Clearly, additional information is required to determine whether garlic and its related compounds can alter the carcinogenicity of direct-acting carcinogens.

Rarely has a comparison of water- and oil-soluble compounds been undertaken. Nevertheless, available evidence suggests that major differences are not likely (Amagase and Milner 1993, Balasenthil et al. 1999, Liu et al. 1992, Schaffer et al. 1996 and 1997, Singh and Shukla 1998). Although subtle differences among garlic preparations can and do occur, quantity rather than source appears to be the key factor influencing the degree of protection (Liu et al. 1992). Variations that surface among preparations likely relate to the content and effectiveness of specific sulfur compounds. More attention must be given to defining the actual active allyl sulfur compounds that bring about the anticancer properties. Clearly, the number of sulfur atoms on the molecule can influence the degree of protection, with diallyl trisulfide > diallyl disulfide > diallyl sulfide (Sakamoto et al. 1997, Sundaram and Milner 1995, Tsai et al. 1996). Similarly, the presence of the allyl group generally enhances protection over that provided by the propyl moiety (Hu et al. 1997, Sundaram and Milner 1995). Overall, bioavailability will be important in determining the overall efficacy of various allyl sulfur compounds as anticancer agents.

TABLE 2

Allyl sulfides with antineoplastic properties

Sulfur compound	Cell type
Ajoene	Lymphocytes, colonic, leukemic
Allicin	Lymphoid
Diallyl sulfide	Prostate, leukocytes
Diallyl disulfide	Lung, colonic, skin, prostate, mammary
Diallyl trisulfide	Lung
S-Allyl cysteine	Neuroblastoma, melanoma
S-Allylmercaptocysteine	Prostate, mammary

Antiproliferative effects of garlic

Cancer is best characterized as uncontrolled proliferating cells. Several lines of evidence point to allyl sulfur compounds as potentially important antitumorigenic agents (Dirsch et al. 1998, Knowles and Milner 1998, Lea and Ayyala 1997, Lea et al. 1999, Li et al. 1995, Pinto et al. 1997, Sakamoto et al. 1997, Scharfenberg et al. 1990 and 1994, Sigounas et al. 1997, Sundaram and Milner 1993 and 1996, Takeyama et al. 1993, Welch et al. 1992). **Table 2** provides a list of some of the allyl sulfur compounds that have been found to alter significantly the proliferation of neoplastic cells. The ability of these compounds to depress tumor cells of different origin suggests that a critical stage in the cancer process is being modified. Active cellular proliferation appears to be a factor in enhancing the growth inhibitory affects ascribed to allyl sulfides (Sigounas et al. 1997). Scharfenberg et al. (1990) found that A549 lung and BJA-B Burkitt lymphoma cells were more than twice as sensitive to the antiproliferative effects of DATS and ajoene than were nonneoplastic MRC-5 lung and FS4/BHK fibroblasts cells. In vivo studies provide evidence that the observations made in vitro have physiologic significance (Riggs et al. 1997, Singh et al. 1996, Sundaram and Milner 1996, Weisberger and Pensky 1958).

Studies from Sundaram and Milner (1996) and Pinto et al. (1997) provide evidence that the allyl group is instrumental in bringing about the growth depression. However, not all allyl sulfides are equal in their ability to reduce tumor proliferation (Pinto et al. 1997, Sundaram and Milner 1993). Studies by Sundaram and Milner 1993 demonstrated that diallyl sulfide, DADS and diallyl trisulfide (DATS) were far more effective in retarding the growth of neoplasms than were water-soluble allyl sulfur compounds such as SAC. Shifts in the cell cycle have been found to correlate with the depression in growth of neoplasms treated with DADS (Knowles and Milner 1998).

The loss of cancer progression after treatment with allyl sulfur compounds likely relates to several epigenetic changes. Two extensively examined mechanisms for epigenetic gene regulation are patterns of DNA methylation and histone acetylations/deacetylations. Several studies indicate that DNA hypermethylation is an important factor involved in the activity of key regulatory genes. DNA methylation and histone acetylation can be modified by enhanced intake of garlic and/or related allyl sulfur compounds. Ludeke et al. (1992) reported that DAS inhibited the formation of O^6-methyldeoxyguanosine in esophagus (26%), nasal mucosa (51%), trachea (68%) and lung (78%) that arose after treatment with *N*-nitrosomethylbenzylamine. Similarly, studies by Lin et al. (1994) and Schaffer et al. (1996) provide evidence that DADS, SAC and deodorized garlic are effective in retarding the DNA methylation caused by NMU. Lea et al. (1999) reported that at least part of the ability of DADS to induce differentiation in DS19 mouse erythroleukemic cells might relate to its ability to increase histone acetylation. Diallyl disulfide caused a marked increase in the acetylation of H4 and H3 histones in DS19 and K562 human leukemic cells, consistent with other studies showing that the disulfide was more effective that the monosulfide. Similar results were also obtained with rat hepatoma and human breast cancer cells. Allyl mercaptan was a more potent inhibitor of histone deacetylase than diallyl disulfide. Interestingly, DADS has been also been reported to inhibit the growth of H-ras oncogene–transformed tumors in nude mice (Singh et al. 1996). This inhibition correlated with the inhibition of p21H-ras membrane association in the tumor tissue. As the molecular targets for allyl sulfur compounds become more evident, it will become easier

to determine who might benefit most from their exaggerated intake.

Diet as a modifier

The effect of garlic and allyl sulfur components on the cancer process cannot be considered in isolation; rather, it is clearly dependent on several environmental and dietary variables. Among the dietary factors are total fat, selenium, methionine and vitamin A (Amagase et al. 1996, Ip et al. 1996, Schaffer and Milner 1997). Amagase et al. (1996) and Ip et al. (1996) reported that selenium supplied either as a component of the diet or as a constituent of the garlic supplement enhanced the protection against 7,12 dimethylbenz[*a*]anthracene (DMBA) mammary carcinogenesis over that provided by garlic alone. Suppression in carcinogen bioactivation, as indicated by a reduction in DNA adducts, may account in part for this combined benefit of garlic and selenium (Schaffer et al. 1997). However, both selenium and allyl sulfur compounds alter cell proliferation and induce apoptosis (Ganther 1999, Knowles and Milner 1998, Sundaram and Milner 1996)

Dietary fatty acid supply can also dramatically influence the bioactivation of DMBA to metabolites capable of binding to rat mammary cell DNA. A significant portion of the enhancement in mammary DNA adducts caused by increasing dietary corn oil consumption can be attributed to linoleic acid intake (Schaffer and Milner 1996). The ability of selected fatty acids to alter DMBA bioactivation may provide clues to a plausible mechanism by which garlic and its allyl sulfur compounds retard chemically induced tumors. As indicated previously, it does not appear that changes in cytochrome P_{450} enzymes account for the protection provided by supplemental garlic, except for the autocatalysis of CYP2E1. During the past decade Smith et al. (1991) found that prostaglandin H synthase could metabolize the bay region diol of benzo(a)pyrene to electrophilic diol epoxides that would bind to DNA. Most recently, our laboratory has reported that DMBA bioactivation appears to depend on cyclooxygenase activity (Schaffer and Milner 1997) and lipoxygenase activity (McGarth and Milner 1999). This evidence is consistent with studies by Ali (1995) that garlic could inhibit cyclooxygenase activity. Studies by McGrath and Milner (1999) provided evidence that both water- and lipid-soluble allyl sulfur compounds could retard the ability of cyclooxygenase and lipoxygenase to bioactivate DMBA. Evidence already existed that lipoxygenase was involved in the bioactivation of several carcinogens (Rioux and Castonguay 1998, Roy and Kulkarni 1999), including benz(a)pyrene (Hughes et al. 1989, Joseph et al. 1994), a carcinogen similar to DMBA. Interestingly, the activation caused by lipoxygenase was ~10 times greater than that caused by cyclooxygenase. Although limited, there are some data indicating that garlic and associated sulfur components can inhibit lipoxygenase activity (Belman et al. 1989). Finally, evidence for the involvement of lipoxygenase in the bioactivation of DMBA comes the data of Song (1999) who reported that feeding the known lipoxygenase inhibitor, nordihydroguaiaretic acid, was accompanied by a marked reduction in DMBA-induced DNA adducts in rat mammary tissue. Collectively, these studies pose interesting questions about the role of both cyclooxygenase and lipoxygenase not only in forming prostaglandins, and therefore modulating tumor cell proliferation and immunocompetence, but also from their involvement in the bioactivation of carcinogens. Clearly, additional attention is warranted to clarify what role, if any, these enzymes have in determining the biological response to dietary garlic.

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